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PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re: Goudsmit et al.

Application Serial No: 09/463,352

Group Art Unit: 1655

Filed: January 21, 2000

Examiner: B. Sisson

For: *NUCLEIC ACID SEQUENCES THAT CAN BE USED AS PRIMERS AND PROBES IN
THE AMPLICATION AND DETECTION OF ALL SUBTYPES OF HIV-1*

MAIL STOP RCE

Commissioner for Patents

P.O. Box 1450

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**DECLARATION OF JAAP GOUDSMIT,
PIETER OUDSHOORN, SUZANNE JURRIAANS
AND VLADIMIR VLADIMIROVICH LUKASHOV
UNDER 37 C.F.R. § 1.131**

Sir:

We, Jaap Goudsmit, Pieter Oudshoorn, Suzanne Jurriaans and Vladimir Vladimirovich Lukashov hereby declare that:

1. We are the inventors of the subject matter of the rejected claims pending in the above-referenced patent application.
2. Prior to June 25, 1997, we conceived and reduced to practice the oligonucleotides having the nucleotide sequence of SEQ ID NOs 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and 11, respectively, as recited in the pending claims, as well as methods of use and kits employing these oligonucleotides to detect HIV-1 nucleic acid in a sample
3. In support of the above statement, we hereby submit as Appendix A a copy of relevant pages of an internal memorandum entitled "Feasability of a qualitative NASBA assay with a broad HIV-1 clade reactivity" prepared by non-inventor, F. Jacobs, under the direction of

group leader and inventor, Pieter Oudshoorn, and submitted to Akzo Nobel. The dates within this document have been blocked out, but are before June 25, 1997.

Specifically, this memorandum shows, in relevant part, with irrelevant text blocked out, on page 4 under section 2.1 entitled "Design of primers and probes," a listing of oligonucleotides identified as P1.1, P1.2, U5 end, P2.1, P2.2, HIV-1 LTR-bio, HIV-LTR-AMN1 and HIV-LTR-AMN2. These oligonucleotides are the oligonucleotides identified in the specification and pending claims as SEQ ID NO:1 and SEQ ID NO:9, SEQ ID NO:2 and SEQ ID NO:10, SEQ ID NO: 3 and SEQ ID NO:12, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7 and SEQ ID NO:8, respectively. The oligonucleotides identified as SEQ ID NOs 1, 2 and 3 are the same as the oligonucleotides identified as SEQ ID NOs 9, 10 and 11, respectively, with the distinction that the latter three oligonucleotides are operably linked to a promoter sequence. (See page 7, lines 13-15 of the specification: "SEQ ID NO 9-11 actually comprise the sequence as reflected by SEQ ID 1-3. In SEQ ID 9-11, the sequences of SEQ ID 1-3 are operably linked to a promoter sequence (the T7 promoter sequence). This makes the sequences especially suitable for use as upstream primer in a transcription based amplification technique such as NASBA.")

The memorandum also shows, in relevant part, with irrelevant text blocked out, on page 5 under section 2.2, entitled "Evaluation and optimization of the primers and probes," a description of a protocol for detecting HIV nucleic acid using the oligonucleotides of this invention. Results obtained by conducting this protocol are shown, in relevant part, with irrelevant text blocked out, on page 10, under section 3.2, entitled "Evaluation of selected primers."

6. In summary, our statements herein and the documents concurrently submitted show conception and reduction to practice of the claimed invention prior to June 25, 1997.

7. We hereby declare that all statements made herein of our own knowledge are true, and that all statements made on information and belief are believed to be true. We further declare that these statements were made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Jaap Goudsmit

Date

Pieter Oudshoorn

Date

Suzanne Jurriaans

Date

Vladimir Vladimirovich Lukashov

Date



Pharma Group

Confidential

SUBJMEMO

Organon Teknika bv

R&D

Boxtel The Netherlands

Diagnostics

8747.970379

K O P I E

Identification:

DMS nr:	8747.970379	Doc. Type:	REPORT	Proj. nr.:	423020
TekName:	SUBJMEMO	Alt. nr.:		Rev. nr.:	00
Title:	Feasibility of a qualitative NASBA assay with a broad HIV-1 clade reactivity.				
Part nrs.:					
Date created:		Supersedes:		DAR/DCO:	
Relevant DMRs:					
Keywords:					

Author:

Department:	Name:	Date:	Signature
NDU	F. Jacobs		

Approval:

Function:	Name:	Date:	Signature
Groupleader	P. Oudshoorn		

To be returned to R&D secretary before submitting for final approval

Final authorization:

Function:	Name:	Date:	Signature
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2. Materials and methods

2.1 Design of primers and probes.

The oligonucleotide sequences are respectively:

P1.1: *aat tct aat acg act cac tat agg g*AG AGG GGC GCC ACT GCT AGA GA
P1.2: *aat tct aat acg act cac tat agg g*AG AGG TTC GGG CGC CAC TGC TAG A
U5 end: *aat tct aat acg act cac tat agg g*CGGGCGCCACTGCTA
P2.1: CTG CTT AAA GCC TCA ATA AA
P2.2: CTC AAT AAA GCT TGC CTT GA

To perform ECL detection one biotin probe and two different detection probes were designed with the following sequences:

HIV-1 LTR-bio: TCT GGT AAC TAG AGA TCC CTC
HIV-LTR-AMN1: TAG TGT GTG CCC GTC TGT.
HIV-LTR-AMN2: AGT GTG TGC CCG TCT GTT.

2.2 Evaluation and optimization of the primers and probes.

The primers were tested directly in the amplification in the combinations P1.1/P2.1, P1.1/P2.2, P1.2/P2.1, P1.2/P2.2 and U5-end/P2.2 on in vitro *LTR* RNA and on Scott Layne RNA (subtype B, stock solution of 5.5×10^9 copies RNA/ml). The input of the RNA was 10^4 copies. The amplifications were examined on a 2% agarose gel and then blotted in 1 hour on zeta probe and cross-linked with UV. The blot was hybridized with the biotin probe (3 μ M) by incubating the blot for 4 hours at 50°C. After hybridization the blot was washed two times for 5 minutes with 3*SSC/1%SDS solution at 50°C and one time for 10 minutes with 2*SSPE/0.1%SDS solution at RT. After this the blot was incubated for 30 minutes with 2 μ l streptavidine/HRP solution (500 U/ml, Enhanced ChemiLuminiscense detection kit from Amersham) in 10 ml 5*SSPE/0.5%SDS. The blot was again washed two times for 5 minutes in 2*SSPE/0.1%SDS solution and one time for 10 minutes in 2*SSPE solution. The blot was dried between tissues and developed with the development solutions from the enhanced chemiluminiscense kit (Amersham). The blot was wrapped in Saran wrap and a film was placed on the blot for a couple of seconds. The film was developed according to the standard procedures.

3.2 Evaluation of selected primers.

Figure 3. Detection of the amplimers on blot.

The primersets used were: nr 1: P1.1-P2.1, nr 2: P1.1-P2.2, nr 3: P1.2-P2.1, nr 4: P1.2-P2.2, nr 5: U5 end-5'LTRSpH1. The RNA used as input were: A: in vitro RNA 10^4 copies per input, B: Scott Layne RNA 10^4 copies per input, C: No Templates.

